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## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PP/WT/PH/3282	<b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/GB99/00966	International filing date (day/month/year) 26/03/1999	Priority date (day/month/year) 27/03/1998
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant ISIS INNOVATION LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.



2. This REPORT consists of a total of 4 sheets, including this cover sheet.

- ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand 27/10/1999	Date of completion of this report 13. 12. 99
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Linker, W Telephone No. +49 89 2399 8703 

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/GB99/00966

**I. Basis of the report**

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

**Description, pages:**

1-10 as originally filed

**Claims, No.:**

1-16 as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:  
☐ the claims, Nos.:  
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

**1. Statement**

Novelty (N)	Yes:	Claims	1-16
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-16
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-16
	No:	Claims	

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/GB99/00966

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2. Citations and explanations

**see separate sheet**

**VIII. Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

**see separate sheet**

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

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International application No. PCT/GB99/00966

**Re Item V**

**Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

The subject-matter of claims 1-16 appears both novel and inventive over the documents cited in the international search report. Thus, the present claims appear to fulfil the requirements of Articles 33(2) and (3) PCT.

The linkage of an allele situated at a locus in a region of chromosome 4 of up to 1 megabase in length, which region contains the locus D4S3032 and /or D4S2921 with asthma appears not to be disclosed or suggested by the prior art, see e.g. ABRAMSON M ET AL: 'The new asthma genetics and its implications for public health' PUBLIC HEALTH REVIEW, vol. 26, no. 2, February 1998 (1998-02), page 138, Table 2, second line.

**Re Item VIII**

**Certain observations on the international application**

Claim 1 should be directed to an "in vitro method" in view of claim 2 and the specification. Claim 12 (page 12, line 15) should read "chromosome 4" in order to comply with Article 6 PCT.

## PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

NOTIFICATION OF THE RECORDING  
OF A CHANGE(PCT Rule 92bis.1 and  
Administrative Instructions, Section 422)

To:

WILKINSON, John, Stephen  
Stevens, Hewlett & Perkins  
1 Augustine's Place  
Bristol BS1 4UD  
ROYAUME-UNI

<b>Date of mailing</b> (day/month/year) 03 February 2000 (03.02.00)	<b>IMPORTANT NOTIFICATION</b>
<b>Applicant's or agent's file reference</b> KP/3282 PCT	
<b>International application No.</b> PCT/GB99/00966	<b>International filing date</b> (day/month/year) 26 March 1999 (26.03.99)

## 1. The following indications appeared on record concerning:

☐ the applicant      ☐ the inventor      ☒ the agent      ☐ the common representative

<b>Name and Address</b> PRIVETT, Kathryn, Louise Stevens, Hewlett & Perkins 1 Serjeants' Inn Fleet Street London EC4Y 1NT United Kingdom	<b>State of Nationality</b>	<b>State of Residence</b>
	<b>Telephone No.</b> 0171 936 2499	
	<b>Facsimile No.</b> 0171 936 2498	
	<b>Teleprinter No.</b>	

## 2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person      ☒ the name      ☒ the address      ☐ the nationality      ☐ the residence

<b>Name and Address</b> WILKINSON, John, Stephen Stevens, Hewlett & Perkins 1 Augustine's Place Bristol BS1 4UD United Kingdom	<b>State of Nationality</b>	<b>State of Residence</b>
	<b>Telephone No.</b> 117 922 6007	
	<b>Facsimile No.</b> 117 922 6009	
	<b>Teleprinter No.</b>	

## 3. Further observations, if necessary:

## 4. A copy of this notification has been sent to:

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<input type="checkbox"/> the International Searching Authority	<input checked="" type="checkbox"/> the elected Offices concerned
<input type="checkbox"/> the International Preliminary Examining Authority	<input type="checkbox"/> other:

<b>The International Bureau of WIPO</b> 34, chemin des Colombettes 1211 Geneva 20, Switzerland  Facsimile No.: (41-22) 740.14.35	<b>Authorized officer</b>  I. Britel  Telephone No.: (41-22) 338.83.38
--	--

## TENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

NOTIFICATION OF THE RECORDING  
OF A CHANGE(PCT Rule 92bis.1 and  
Administrative Instructions, Section 422)

To:

WILKINSON, John, Stephen  
Stevens, Hewlett & Perkins  
1 Augustine's Place  
Bristol BS1 4UD  
ROYAUME-UNIDate of mailing (day/month/year)  
19 April 2000 (19.04.00)Applicant's or agent's file reference  
KP/3282 PCT

## IMPORTANT NOTIFICATION

International application No.  
PCT/GB99/00966International filing date (day/month/year)  
26 March 1999 (26.03.99)

## 1. The following indications appeared on record concerning:

☐ the applicant ☐ the inventor ☒ the agent ☐ the common representative

## Name and Address

PENNANT, Pyers  
Stevens, Hewlett & Perkins  
1 Serjeants' Inn  
Fleet Street  
London EC4Y 1NT  
United Kingdom

## State of Nationality

## State of Residence

## Telephone No.

+44-171-936-2499

## Facsimile No.

+44-171-936-2498

## Teleprinter No.

## 2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☐ the name ☒ the address ☐ the nationality ☐ the residence

## Name and Address

PENNANT, Pyers  
Stevens, Hewlett & Perkins  
Halton House  
20/23 Holborn  
London EC1N 2JD  
United Kingdom

## State of Nationality

## State of Residence

## Telephone No.

+44 20 7404 1955

## Facsimile No.

+44 20 7404 1844

## Teleprinter No.

## 3. Further observations, if necessary:

## 4. A copy of this notification has been sent to:

☒ the receiving Office ☐ the designated Offices concerned  
☐ the International Searching Authority ☒ the elected Offices concerned  
☐ the International Preliminary Examining Authority ☐ other:The International Bureau of WIPO  
34, chemin des Colombettes  
1211 Geneva 20, Switzerland

## Authorized officer

Anman QIU

Facsimile No.: (41-22) 740.14.35

Telephone No.: (41-22) 338.83.38

## PATENT COOPERATION TREATY

PCT

## NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents  
 United States Patent and Trademark  
 Office  
 Box PCT  
 Washington, D.C.20231  
 ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year)

02 December 1999 (02.12.99)

International application No.

PCT/GB99/00966

Applicant's or agent's file reference

KP/3282 PCT

International filing date (day/month/year)

26 March 1999 (26.03.99)

Priority date (day/month/year)

27 March 1998 (27.03.98)

Applicant

COOKSON, William, Osmond, Charles, Michael et al

1. The designated Office is hereby notified of its election made:



in the demand filed with the International Preliminary Examining Authority on:

27 October 1999 (27.10.99)



in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was

was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO  
 34, chemin des Colombettes  
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Authorized officer

J.M. Vivet

Telephone No.: (41-22) 338.83.38

## PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

NOTIFICATION OF THE RECORDING  
OF A CHANGE(PCT Rule 92bis.1 and  
Administrative Instructions, Section 422)

To:

CORNISH, Kristina  
Kilburn & Strode  
20 Red Lion Street  
London WC1R 4PJ  
ROYAUME-UNI

Date of mailing (day/month/year)

29 September 2000 (29.09.00)

Applicant's or agent's file reference

KP/3282 PCT

## IMPORTANT NOTIFICATION

International application No.

PCT/GB99/00966

International filing date (day/month/year)

26 March 1999 (26.03.99)

1. The following indications appeared on record concerning:

☐

the applicant

☐

the inventor

☒

the agent

☐

the common representative

Name and Address

WILKINSON, John, Stephen  
Stevens, Hewlett & Perkins  
1 Augustine's Place  
Bristol BS1 4UD  
United Kingdom

State of Nationality

State of Residence

Telephone No.

117 922 6007

Facsimile No.

117 922 6009

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☒

the person

☐

the name

☐

the address

☐

the nationality

☐

the residence

Name and Address

CORNISH, Kristina  
Kilburn & Strode  
20 Red Lion Street  
London WC1R 4PJ  
United Kingdom

State of Nationality

State of Residence

Telephone No.

020 7539 4200

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3. Further observations, if necessary:

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The International Bureau of WIPO  
34, chemin des Colombettes  
1211 Geneva 20, Switzerland

Authorized officer

Jean-Marie McAdams

Facsimile No.: (41-22) 740.14.35

Telephone No.: (41-22) 338.83.38



## PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

To:

CORNISH, Kristina  
Kilburn & Strode  
20 Red Lion Street  
London WC1R 4PJ  
ROYAUME-UNI

**NOTIFICATION OF THE RECORDING  
OF A CHANGE**

(PCT Rule 92bis.1 and  
Administrative Instructions, Section 422)

<b>Date of mailing</b> (day/month/year) 31 October 2000 (31.10.00)	<b>IMPORTANT NOTIFICATION</b>
<b>Applicant's or agent's file reference</b> KP/3282 PCT	
<b>International application No.</b> PCT/GB99/00966	<b>International filing date</b> (day/month/year) 26 March 1999 (26.03.99)

## 1. The following indications appeared on record concerning:

☒ the applicant ☐ the inventor ☐ the agent ☐ the common representative

<b>Name and Address</b> ISIS INNOVATION LIMITED 2 South Parks Road Oxford OX1 3UB United Kingdom	<b>State of Nationality</b> GB	<b>State of Residence</b> GB
	<b>Telephone No.</b>	
	<b>Facsimile No.</b>	
	<b>Teleprinter No.</b>	

## 2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☐ the name ☒ the address ☐ the nationality ☐ the residence

<b>Name and Address</b> ISIS INNOVATION LIMITED Ewert House Ewert Place Summertown Oxford OX2 7BZ United Kingdom	<b>State of Nationality</b>	<b>State of Residence</b>
	<b>Telephone No.</b>	
	<b>Facsimile No.</b>	
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## 3. Further observations, if necessary:

## 4. A copy of this notification has been sent to:

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<b>The International Bureau of WIPO</b> 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	<b>Authorized officer</b> Peggy Steunenberg Telephone No.: (41-22) 338.83.38
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**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12Q 1/68</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 99/50449</b> <b>(43) International Publication Date:</b> 7 October 1999 (07.10.99)
<b>(21) International Application Number:</b> PCT/GB99/00966 <b>(22) International Filing Date:</b> 26 March 1999 (26.03.99)  <b>(30) Priority Data:</b> 9806653.3 27 March 1998 (27.03.98) GB  <b>(71) Applicant (for all designated States except US):</b> ISIS INNOVATION LIMITED [GB/GB]; 2 South Parks Road, Oxford OX1 3UB (GB).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> COOKSON, William, Osmond, Charles, Michael [GB/GB]; 67 Hilltop Road, Oxford OX4 1PD (GB). BHATTACHARYYA, Sumit [GB/GB]; 20 Bury Road, Stapleford, Cambridgeshire CB2 5BP (GB). LEAVES, Nicholas [GB/GB]; 47 Burdell Avenue, Sandhills, Oxford OX3 8EE (GB).  <b>(74) Agent:</b> PRIVETT, Kathryn, Louise; Stevens, Hewlett & Perkins, 1 Serjeants' Inn, Fleet Street, London EC4Y 1NT (GB).		<b>(81) Designated States:</b> AU, CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> POLYMORPHISM II: LINKAGE OF ASTHMA TO A LOCUS ON CHROMOSOME 4  <b>(57) Abstract</b>  A method for diagnosing an individual as being asthmatic, or as having a predisposition to asthma is described, which method comprises demonstrating in the individual the presence or absence of one or more alleles which are associated with asthma, wherein the one or more alleles are situated at a locus in a region of chromosome 4 of up to 1 megabase in length, which region contains the locus D4S3032 and/or D4S2921.		

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## POLYMORPHISM II: LINKAGE OF ASTHMA TO A LOCUS ON CHROMOSOME 4

This invention is concerned with methods for the diagnosis of asthma and with materials and methods relating thereto.

5           Asthma is a disease which is becoming more prevalent and is the most common disease of childhood (1). Most asthma in children and young adults is initiated by IgE mediated allergy (atopy) to inhaled allergens such as house dust mite and cat dander. However, not all asthmatics are atopic, and most atopic individuals do not have asthma, so that factors in  
10 addition to atopy are necessary to induce the disease (2,3). Asthma is strongly familial, and is due to the interaction between genetic and environmental factors. The genetic factors are thought to be variants of normal genes ("polymorphisms") which alter their function to predispose to asthma.

15           Asthma may be identified by recurrent wheeze and intermittent air flow limitation. An asthmatic tendency may be quantified by the measurement of bronchial hyper-responsiveness in which an individual's dose-response curve to a broncho-constrictor such as histamine or methacholine is constructed. The curve is commonly summarised by the dose  
20 which results in a 20% fall in air flow (PD20) or the slope of the curve between the initial air flow measurement and the last dose given (slope). Asthma is accompanied by blood eosinophilia, and eosinophils are prominent in asthmatic airways.

          In the atopic response, IgE is produced by B-cells in response to  
25 allergen stimulation. These antibodies coat mast cells by binding to the high affinity receptor for IgE (FcεRI). When a multivalent allergen binds to an IgE-coated mast cell, the cross-linking of adjacent IgEs by allergen initiates a series of cellular events leading to the destabilisation of the cell membrane

and release of inflammatory mediators. This results in mucosal inflammation, wheezing, coughing, sneezing and nasal blockage.

Atopy can be diagnosed by (i) a positive skin prick test in response to a common allergen; (ii) detecting the presence of specific serum  
5 IgE for allergen; or (iii) by detecting elevation of total serum IgE.

Genetic factors underlying a disease may be identified through localisation to particular chromosomal regions by genetic linkage. Genetic linkage is established by the study of families. It relies on matching the inheritance of disease with genetic polymorphisms of known localisation  
10 (known as "genetic markers"). In a complex disease such as asthma, genetic linkage will typically localise genes to within 10 - 20 Megabases (Mb) of DNA. A region of this size may contain 350 - 700 genes, and will be too large to permit immediate identification of the disease-causing gene.

Closer localisation of disease-causing genes may be  
15 accomplished by the detection of associations between particular alleles and the disease phenotype. Over short segments of DNA, distinctive alleles of the individual polymorphisms will show non-random association with alleles of neighbouring polymorphisms. This phenomenon, known as "linkage disequilibrium" occurs over 50-500 Kilobases (Kb) of DNA. Linkage  
20 disequilibrium may be detected by the study of individuals as well as by the study of families.

Disease-causing alleles will be in linkage disequilibrium with non-functional polymorphisms from the same chromosomal segment. It is therefore possible to detect allelic association with disease from particular  
25 chromosomal segments, without identifying the exact polymorphism and gene underlying the disease state.

The detection of allelic association may therefore give information as to disease susceptibility in a particular individual. Furthermore,

allelic association is indicative of a disease-causing gene being present within 500 Kb of DNA in either direction from the allele (i.e. 1 Mb in total). Such a region may contain only 30 genes, within which the identification of the disease-causing gene is possible.

5           The presence of linkage disequilibrium also means that other polymorphisms may be anticipated to associate with disease, and that these additional polymorphisms will also be diagnostic of disease susceptibility in particular individuals.

Genetic associations with atopy have been demonstrated.  
10       WO 95/05481 discloses that variants of the gene encoding the  $\beta$ -subunit of the high-affinity receptor for IgE (Fc $\epsilon$ RI $\beta$ ) are associated with atopy. It teaches a method for diagnosing atopy which is based upon the demonstration of the presence or absence of one of two variants in a specific portion of the DNA sequence of the gene encoding Fc $\epsilon$ RI $\beta$ , located near the  
15       commencement of exon 6 of the Fc $\epsilon$ RI $\beta$  gene on chromosome 11. A further variant has also been found in which the unusual variant sequence is in the coding sequence for the C-terminal cytoplasmic tail of Fc $\epsilon$ RI $\beta$  (4).

Tumour Necrosis Factor (TNF) is a pro-inflammatory cytokine that is found in increased concentration in asthmatic airways (5). We have  
20       previously shown that polymorphisms within the TNF gene are associated with an increased risk of asthma (6).

The known polymorphisms do not account for all of the genetic factors which predispose to asthma. In particular, asthma is not necessarily an atopic disease. Identification of further genetic polymorphisms linked to  
25       asthma will allow the identification of children at risk of asthma before the disease has developed (for example immediately after birth), with the potential for prevention of disease. The presence of particular polymorphisms may predict the clinical course of disease (e.g. severe as opposed to mild) or the

response to particular treatments. This diagnostic information will be of use to the health care, pharmaceutical and insurance industries.

We have previously established linkage of bronchial hyper-responsiveness to chromosome 4 (8). However, this finding is of no use in  
5 diagnosis.

It has now been discovered that a genetic polymorphism known as D4S3032\*5 on chromosome 4 and a nearby polymorphism known as D4S2921\*13 are associated with asthmatic traits. Specifically, D4S3032\*5 is associated with bronchial hyper-responsiveness and D4S2921\*13 is  
10 associated with peripheral eosinophilia, both of these being traits which underlie asthma. The two polymorphisms can therefore be used as diagnostic tools.

The invention therefore provides a method for diagnosing an individual as being asthmatic, or as having a predisposition to asthma, which  
15 method comprises demonstrating in the individual the presence or absence of one or more alleles which are associated with asthma, wherein the one or more alleles are situated at a locus in a region of chromosome 4 of up to 1 megabase in length, which region contains the locus D4S3032 and/or D4S2921.

20 The 1Mb region of chromosome 4 referred to flanks the D4S3032 and D4S2921 loci. Thus, the specific allele D4S3032\*5, or D4S2921\*13, or other unusual polymorphisms in the region which are associated with asthma, may be the subject of identification in the method according to the invention. Equally two or more such alleles may be the  
25 subject of identification, including in particular the combination of D4S3032\*5 and D4S2921\*13.

Current diagnostic methods involving detection at the nucleic acid level normally comprise the steps of:

- (i) obtaining a suitable tissue sample from the individual;
- (ii) preparing from the tissue sample a nucleic acid sample;
- (iii) analysing the nucleic acid sample for the presence or absence of the relevant nucleic acid sequence, such as a specific allele.

5

Preferably, an amplification step is performed prior to the analysis, such that the locus at which the allele is situated is amplified. A preferred amplification technique is the PCR, although any suitable method of nucleic acid amplification may be employed.

10

In further aspects, the invention provides a pair of oligonucleotide primers for amplification of an allele which is associated with asthma, which allele is situated at a locus in a region of chromosome 4 of up to 1 megabase in length, which region contains the locus D4S3032 and/or D4S2921; and an assay kit comprising the pair of oligonucleotide primers.

15

The specific allele for identification may take the form of microsatellite repeats, which are nucleotide sequences containing short, repeated nucleotide motifs, usually a dinucleotide or a trinucleotide motif. A pair of primers which hybridize under suitably stringent conditions, to sequences at a position on either side of the microsatellite repeats, may be used to amplify the microsatellite repeats by PCR. Differences in the number of repeats are recognised by size differences in the PCR products. An allele which has a specified number of repeats and therefore a known size can thus be identified. D4S3032\*5 and D4S2921\*13 are examples of such alleles.

20

The primers employed in the method comprise nucleic acid sequences which are complementary to, or substantially complementary to unique sequences either side of the microsatellite repeats, such that only the relevant polymorphic region of the genome is amplified. The conditions under which the amplification is performed are gauged such that specific

25



hybridization of the primers to the flanking sequences occurs and non-specific hybridization is avoided. The hybridization conditions are suitably stringent for that purpose. Standard techniques can be used to identify an appropriate set of reaction conditions.

5                   Typically, the PCR products are detected by means of a detectable label attached to one of the PCR primers. Alternatively another form of labeling may be used such as a labeled sequence specific probe which hybridizes to the amplified sequences. The label may be a fluorescent or other label. The PCR products are subjected to size determination,  
10 typically involving size-separation for example by gel electrophoresis, and the presence or absence of the allele of interest is determined.

                  It will be evident that the invention is not limited with regard to the manner in which the presence or absence of the allele of interest is determined. The labeling, detection, separation or any other aspect of the  
15 method as described here may be replaced by other suitable known techniques and reagents.

                  The allele for identification may be an allele other than D4S3032\*5 or D4S2921\*13 which is in linkage disequilibrium with D4S3032\*5 or D4S2921\*13 and is associated with asthma. This includes alleles of both  
20 functional and non-functional polymorphisms. Functional polymorphisms include polymorphisms within genes, usually within coding sequences of genes. Non-functional polymorphisms are polymorphisms which do not themselves cause the disease.

                  This invention will now be further described in the Examples  
25 section which follows. The Examples are intended to be illustrative and do not limit the scope of the invention in any way.

## EXAMPLES

### Description of Laboratory Testing

#### **Subjects**

Two panels of subjects have been studied.

5            Panel A consisted of 80 nuclear families sub-selected from an Australian population sample of 230 families (8). The panel contained a total of 203 offspring forming 172 sib-pairs. 12% of the children were asthmatic.

Panel C consisted of 87 nuclear families recruited through a child attending an asthma clinic in the Oxford region. The families contained  
10   216 offspring (148 sibling pairs), of whom 44% were asthmatic.

#### **Phenotyping**

            Bronchial responsiveness to methacholine was measured as previously described (8): the maximum dose administered was 12  $\mu$ mol. The  
15   slope of the dose-response curve was calculated as (pre-dose forced expiratory volume in one second (FEV1) - last FEV1)  $\div$  the cumulative dose of methacholine). A constant of 0.01 was added to each measurement, to allow loge transformation when Slope was  $\leq 0$ . Eosinophils in peripheral blood were  
Coulter-counted and the values loge transformed before analysis.

20

#### **Genotyping**

            The microsatellite markers D4S3032 and D4S2921 were typed by semi-automated fluorescent methods, as described previously (8). These markers are in close proximity at the telomeric region of the long arm of  
25   chromosome 4.

            The polymerase chain reaction primer sequences for the markers were as follows:

D4S3032 5' TGA AAT TCT ATT GAC CAA TGA TGT G (SEQ ID NO: 1)

UD4S3032 5' TAG CAC CTG GAT TTA CCA TGA C (SEQ ID NO: 2)

D4S2921 5' TCC TTC AGG AAC TGG TG (SEQ ID NO: 3)

UD4S2921 5' TTA AAA ATC TAC AGA CAA GGG C (SEQ ID NO: 4)

5 The polymerase chain reaction conditions were as follows: The reaction volumes were 10 $\mu$ l, containing 50ng of genomic DNA, 200mM dNTPs, 1 x NH<sub>4</sub><sup>+</sup> buffer, 50ng oligonucleotide primers (forward labelled fluorescently), 0.5 to 3.0mM MgCl<sub>2</sub> and 0.2U Taq polymerase. Cycling conditions were 1 min at 95°C, 1 min at 55°C and 45s at 72°C; 28 cycles were  
10 used. PCRs were performed on an Hybaid Omnigene thermal cycler.

Electrophoresis and allele scoring were as follows:

PCR products were mixed with a size standard (GS350 TAM) in loading buffer (80% (v/v) formamide, 20% (v/v) 50mM EDTA, 0.1% (w/v) blue dextran). Samples were denatured at 95°C for 4 min immediately prior to loading onto a  
15 6% polyacrylamide gel and were electrophoresed at 800v for 6h on an Applied Biosystems (ABI) 313 DNA sequencer. Allele sizes were assigned using the ABI GENESCAN and ABI GENOTYPER software.

### ***Association Analysis***

20 Association was tested against the phenotype of bronchial hyper-responsiveness and peripheral blood eosinophil counts by the ASSOC routine of the SAGE (ver2.2) computer program.

### **Results**

25

#### ***Association with D4S3032 allele 5 and D4S2921 allele 13***

Each of the markers was then tested for association with the asthma phenotype. Association was seen in panel A for allele 5 of D4S3032

(D4S3032\*5) and bronchial hyper-responsiveness. This allele is 145 base pairs in size, using the primers described above. Association between allele 13 of D4S2921 (D4S2921\*13) and eosinophil counts were seen in both panels. This allele is 162 base pairs in size, using the primers described above. (Other suitable primers can be designed and their amplification product size determined for D4S3032\*5 or D4S2921\*13, using known sequence information (9).) The results of testing were as follows:

Trait	Marker	Allele	Panel A		Panel C		Combined	
			$\chi^2$	p	$\chi^2$	p	$\chi^2$	p
Slope	d4s3032	5	13.56	0.0002	-	-	-	-
Eosinophils	d4s2921	13	5.00	0.03	11.17	0.0008	17.61	0.0000

The recombination fraction between D4S3032 and D4S2921 was 3%, indicating in this telomeric region that the distance between the markers is of the order of 0.5 to 1 megabase.

The results indicate that D4S3032\*5 and D4S2921\*13 show strong association with intermediate phenotypes underlying asthma in two diverse panels of subjects. It may therefore be inferred that a gene influencing asthma is present within 500 kilobases in either direction of D4S3032 and D4S2921.

## REFERENCES

1. Strachan, D.P., Anderson, H.R., Limb, E.S., O'Neill, A. and Wells, N. (1994). A national survey of asthma prevalence, severity, and treatment in Great Britain. *Arch. Dis. Child.*, **70**, 174-178.

2. Fraser, R.S., Paré, J.A.P., Fraser, R.G. and Paré, P.D., eds. (1994) Synopsis of Diseases of the Chest. WB Saunders Company, Philadelphia: 635-53.
3. Djukanovic, R., Roche, W.R., Wilson, J.W., Beasley, C.R., Twentyman, O.P., Howarth, R.H. and Holgate S.T. (1990) Mucosal inflammation in  
5 asthma. *Am. Rev. Respir. Dis.*, **142**, 434-457.
4. Hill, M.R. and Cookson, W.O.C.M. (1996). A new variant of the  $\beta$  subunit of the high-affinity receptor for Immunoglobulin E (Fc $\epsilon$ RI- $\beta$  E237G): Associations of measures of atopy and bronchial hyper-responsiveness. *Hu.*  
10 *Mol. Gen.* **5**, 959-962.
5. Broide, D.H., Lotz, M., Cuomo, A.J., Coburn, D.A., Federman, E.C. and Wasserman, S.I. (1992) Cytokines in symptomatic asthma airways. *J. Allergy Clin. Immunol.* **89**, 958-967.
6. Moffatt M.P, Cookson WOCM. Tumour Necrosis Factor Haplotypes.  
15 and Asthma. *Hum Molec Genet* 1997; **6**: 551-554.
7. Bailly S, di Giovine FS, Blakemore A.I.F, Duff G.W. Genetic polymorphism of human interleukin-1 $\alpha$ . *Eur J Immunol* 1993; **23**: 1240-1245.
8. Daniels S.E, Bhattacharyya S, James A, Leaves N.I, Young A, Hill M.R, Faux J.A, Ryan G.F, le Söuef P.N, Lathrop G.M, Musk A.W, Cookson  
20 W.O.C.M. A genome-wide search for quantitative trait loci underlying asthma. *Nature* 1996; **383**: 247-250.
9. Dib S. et al. A comprehensive map of the human genome based on 5264 microsatellites. *Nature* 1996; **380**: 152-154.

## CLAIMS

1. A method for diagnosing an individual as being asthmatic, or as having a predisposition to asthma, which method comprises demonstrating in  
5 the individual the presence or absence of one or more alleles which are associated with asthma, wherein the one or more alleles are situated at a locus in a region of chromosome 4 of up to 1 megabase in length, which region contains the locus D4S3032 and/or D4S2921.
2. The method according to claim 1, wherein the method comprises  
10 the steps of:
  - (i) obtaining a suitable tissue sample from the individual;
  - (ii) preparing from the tissue sample a nucleic acid sample;
  - (iii) analysing the nucleic acid sample for the presence or absence of the allele.
- 15 3. The method according to claim 2, wherein prior to analysis, the locus at which the or each allele is situated is amplified.
4. The method according to claim 3, wherein the amplification is by the PCR.
5. The method according to any one of claims 1 to 4, wherein the  
20 locus at which the or each allele is situated comprises microsatellite repeats of variable length.
6. The method according to claim 3 or claim 4, wherein the amplification is performed using a pair of primers for each allele, wherein each primer in a pair hybridises under suitably stringent conditions to a region either  
25 side of the microsatellite repeats.
7. The method according to any one of claims 1 to 6, wherein the allele for identification is D4S3032\*5.

8. The method according to any one of claims 1 to 6, wherein the allele for identification is D4S2921\*13.

9. The method according to any one of claims 1 to 6, wherein the alleles for identification are D4S3032\*5 and D4S2921\*13.

5 10. The method according to any one of claims 3 to 9, wherein the analysis is carried out by size separation of amplification products.

11. The method according to claim 10, wherein the primers in the pair of primers comprise the oligonucleotide sequences identified by SEQ ID NO: 1 and SEQ ID NO: 2 or substantially similar sequences, for D4S3032\*5; or identified by SEQ ID NO: 3 and SEQ ID NO: 4 or substantially similar sequences, for D4S2921\*13; or both of the aforementioned pairs of primers for both of the aforementioned alleles.

12. A pair of oligonucleotide primers for amplification of an allele which is associated with asthma, which allele is situated at a locus in a region of chromosome 2 of up to 1 megabase in length, which region contains the locus D4S3032 and/or D4S2921.

13. The pair of oligonucleotide primers according to claim 12, one of which is labeled with a detectable marker.

14. The pair of oligonucleotides according to claim 12 or claim 13, capable of hybridising under suitably stringent conditions to a region either side of a region of microsatellite repeats at D4S3032 or D4S2921.

15. The pair of oligonucleotide primers according to claim 14, comprising the oligonucleotide sequences identified by SEQ ID NO:1 and SEQ ID NO:2 or substantially similar sequences, for D4S3032\*5; or the oligonucleotide sequences identified by SEQ ID NO: 3 and SEQ ID NO:4 or substantially similar sequences, for D4S2921\*13.

16. An assay kit which comprises the pair of oligonucleotide primers according to any one of claims 12 to 15.

## SEQUENCE LISTING

<110> Isis Innovation Limited

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22

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/GB 99/00966

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MARONE G: "Asthma: recent advances" TRENDS IMMUNOLOGY TODAY, vol. 19, no. 1, January 1998 (1998-01), pages 1-5, XP004101456 page 1, paragraph 2 ---	1
A	ABRAMSON M ET AL: "The new asthma genetics and its implications for public health" PUBLIC HEALTH REVIEW, vol. 26, no. 2, February 1998 (1998-02), pages 127-144, XP002110731 see abstract and page 137, para 4 to page 138 --- -/--	1-16

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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"P" document published prior to the international filing date but later than the priority date claimed

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"&" document member of the same patent family

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Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Osborne, H

# INTERNATIONAL RESEARCH REPORT

International Application No

PCT/GB 99/00966

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DANIELS S ET AL: "A genome-wide search for quantitative trait loci underlying asthma " NATURE, vol. 383, 1996, pages 247-50, XP002110730 cited in the application the whole document ----	1-16
A	WO 95 05481 A (ISIS INNOVATION LTD.) 23 February 1995 (1995-02-23) cited in the application the whole document ----	1-16
A	DIB C ET AL: "A comprehensive genetic map of the human genome based on 5264 microsatellites" NATURE, vol. 380, 14 March 1996 (1996-03-14), pages 152-154, XP002110732 -----	



PCT/GB 99/00966

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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